

MONITORING THE POST VACCINAL EFFECT OF EXPERIMENTALLY ATTENUATED LUMPY SKIN DISEASE VACCINE IN PREGNANT COWS

By

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ABSTRACT

In this study the immune response of pregnant cattle vaccinated with an experimentally prepared tissue culture live attenuated Lumpy Skin Disease virus (LSDV) vaccine was determined. Eight pregnant cows were vaccinated intradermal (I/D) with the field dose ($10^{3.0}$ TCID₅₀/animal) of the prepared LSD vaccine, 2 pregnant cows were vaccinated I/D with 10x of field dose of LSD vaccine ($10^{4.0}$ TCID₅₀) for safety and 2 pregnant cows kept unvaccinated as control. All vaccinated and unvaccinated control pregnant cows were kept under clinical examination in insect proof rooms. Body temperature and post vaccinal reaction were recorded daily for 14 days. Vaccinated cows showed slight rise in body temperature started on the 4th day post vaccination (PV) and returned to the normal temperature on the 9th day PV. Local reaction appeared at the inoculation site on the 4th day as a small size round firm nodule and persists for 12-15 days PV. Serum samples were collected from vaccinated cows showed protective level of LSD antibodies (1.5) and (1.32) for NI and ELISA SP ratio at the second week post vaccination and increased gradually till reached the maximum (3.25) NI and (2.35) S/P at 4th week post vaccination, respectively. SNT and ELISA carried out on collected serum samples from newly born calves born to vaccinated dams showed NI (2.25) and SP ratio (1.86) at the first week of age and persisted in protective level (1.5 NI and 1.11 S/P) until 6 months post parturition, then decreased and be lower than protective level. In conclusion, this study revealed that LSD live attenuated vaccine is highly immunogenic and safe as did not cause abortion nor LSD signs appeared in vaccinated pregnant cows, no teratogenic effect or congenital abnormalities in newborn calves and inducing a high level of antibody titer which gave protective maternal immunity persisted up to 6 months.

INTRODUCTION

Lumpy skin disease is an acute, subacute or chronic generalized eruptive viral skin disease affecting cattle of all ages and caused by Capripox virus which is antigenically related to sheep and goat pox virus (**Micheal et al., 1994**). Infected animals suffers from fever, depression, anorexia, decreased body weight, excessive salivation, rhinitis, conjunctivitis and cutaneous nodules that could be seen anywhere on the body, as well as some cattle may have swollen lymph nodes. The disease causes reduction in milk yield of lactating cattle and abortions in pregnant cows (**Abutarbush et al. 2014**). Animals recover slowly from the severe disease and may suffer from mastitis, pneumonia, formation of necrotic skin plugs leaving deep holes in the hide (**Tuppurainen et al., 2011**). Sheep pox virus (SPV) vaccines have been widely used for immunizing cattle against lumpy skin disease virus. In the Middle East and the Horn of Africa, these vaccines have been associated with incomplete protection (**Caroline et al. 2014**). In Egypt, the control of LSD was done by vaccination of cattle with SPV using Romanian and Kenyan strains (**Michael et al., 1994 and 1997, Yasser et al., 2015**). The protection percent reached 67% at 23 weeks post vaccination with SPV under field conditions and the maternal immunity disappears after 4 months as calves should be vaccinated at this age (**Ayatollah et al., 2015**). Homologous vaccines are more effective than sheep pox strain vaccines and the most effective for prevention of the disease. Field evidence about vaccine efficacy is reported from Jordan, Greece and Turkey (**Abutarbush, 2016 and Sevik and Dogan 2016**). The vaccination against LSD with LSD vaccine remains a viable method for control the LSD; it appears to reduce morbidity and mortality rates, production loss and treatment cost (**Abutarbush et al., 2014**). The immune response of vaccinated cattle with SPV or LSD vaccine against LSD has been evaluated by using SNT and ELISA (**AboulSoud et al., 2004, Christine et al., 2014 and Yasser et al., 2015**). Maternal antibodies those transfer through placenta and colostrum are important mechanism that protects the offspring from infections in early life while its humoral response is inefficient (**Hurley and Theil 2011**). This study aims to evaluate the post vaccinal (PV) effect and acquired immunity for attenuated LSD vaccine in pregnant cows and the maternal immunity for their newborn calves was determined.

MATERIAL AND METHODS

Live attenuated Lumpy skin disease vaccine:

Live attenuated LSD experimentally prepared vaccine (Ismalia strain, **House *et al.* 1990**) with titer $10^{5.2}$ TCID₅₀ was supplied by Pox Vaccines Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt.

Madin Derby Bovine kidney Cell (MDBK) culture:

It was kindly supplied by Pox Vaccines Research Department, VSVRI, cultured by using Earl's minimum essential medium (MEM) with neonatal calf serum (10% for growth medium and 2% for maintenance medium, **(OIE, 2012)**).

Animals:

Twelve susceptible pregnant cows (eight month pregnancy) were housed in insect proof isolated units and screened for their freedom from LSD antibodies. Those animals were vaccinated and observed for post vaccinal reaction and serum samples were collected for monitoring the (PV) immune response. The offspring calves were clinically examined and their serum samples were collected to examine for their maternal immunity.

Animal vaccination:

Field dose vaccination.

Vaccination of 8 pregnant cows was performed as described by **Daoud *et al.* (1998)**, as the field dose of tissue culture live attenuated LSD vaccine (10^3 TCID₅₀/dose) inoculated intradermal (I/D) and 2 pregnant cows were kept in contact as control without vaccination.

Safety test of the prepared vaccine:

The experimental prepared LSD vaccine was tested for its safety by inoculation of two pregnant cows I/D with 10x of the field dose of LSD vaccine to check its safety as previously described **(OIE, 2012)**.

Rabbit Hyperimmune Serum (HIS) for LSDV:

LSD hyperimmune sera were prepared in Bosket rabbits (2-3 kg body weight according to method described by **Babiuk *et al.* (2009)** and used as positive control in SNT and ELISA.

Serum Samples:

Serum samples were collected from vaccinated pregnant cows till parturition and follow up till the weaning time (weekly for 24 weeks) and from their offspring calves (at the 1st week after parturition and then monthly for 7 months) and stored at -20°C till application of ELISA and SNT.

Evaluation of humeral immunity:

Serum Neutralization Test (SNT).

All collected sera were screened against LSD according to the method described by OIE (2012), the neutralizing index (NI) was calculated according to Reed and Muench (1938).

Indirect ELISA:

All collected sera were screened against LSD by indirect ELISA using ID Vet Capripox ELISA Kit, France, according to the method described by Babiuk *et al.* (2009).

RESULTS

Safety and Clinical effect of vaccinated pregnant cow by LSD vaccine.

As shown in (Table 1) and Fig.(1), The post vaccinal reaction showed rise in body temperature started on the 4th day as 38.8°C and reached 39.3°C on the 7th day, then began to decline and returned to the normal temperature (38.1°C) by the 9th day post inoculation, the dams temperature were recorded and clarified in (Table 1). No abortion or generalized LSD reaction and no teratogenic or congenital abnormalities were founded in the newborn offspring calves recorded in vaccinated pregnant cows either by field dose or 10x field dose. On the other hand vaccinated cows showed local reaction appeared at the inoculation site on the 4th day as a small size round firm nodule (Photo 1) and persist for 12-15 days before hiding.

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Table (1): Mean of daily records of body temperature of vaccinated pregnant cows.

Time post vaccination	Vaccinated pregnant cows	Non-vaccinated Control
0	37.9 ± 0.1	38.1
1st	38.1 ± 0.1	38.0
2nd	38.2 ± 0.2	37.9
3rd	38.1 ± 0.4	38.1
4th	38.8 ± 0.1	38.1
5th	38.9 ± 0.1	38.0
6th	39.1 ± 0.1	37.9
7th	39.3 ± 0.1	38.1
8th	38.7 ± 0.1	38.1
9th	38.1 ± 0.1	37.9
10th	38.2 ± 0.1	37.9
11th	38.1 ± 0.1	38.0
12th	38.2 ± 0.1	37.9
13th	38.1 ± 0.5	38.1
14th	38.1 ± 0.2	38.1

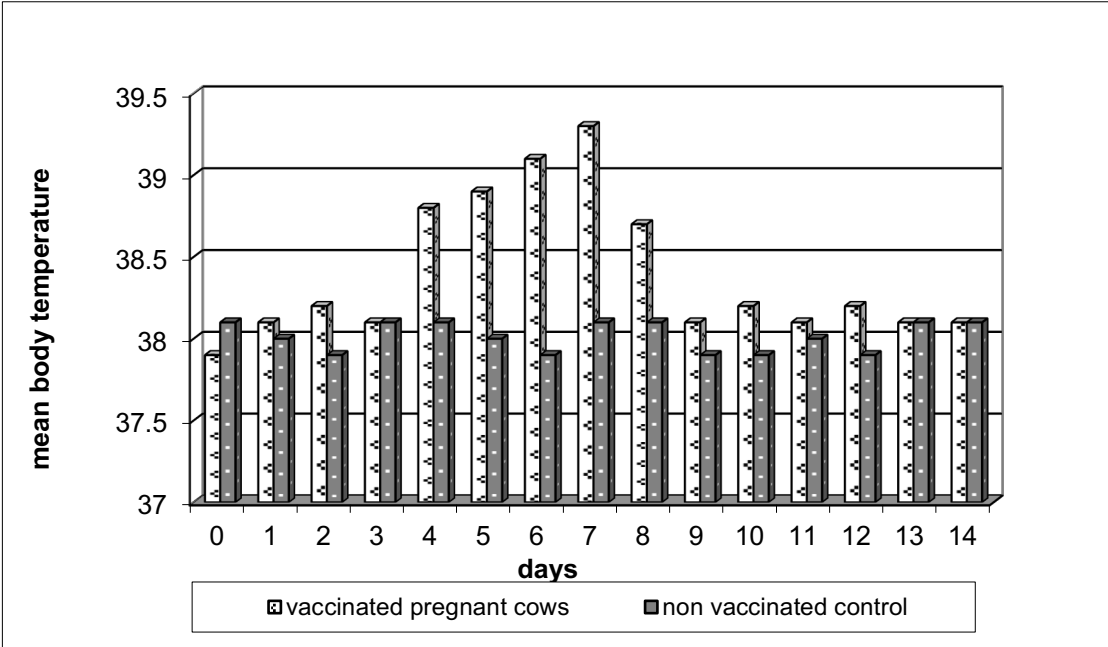


Fig. (1): Mean of daily records of body temperature of vaccinated pregnant cows.

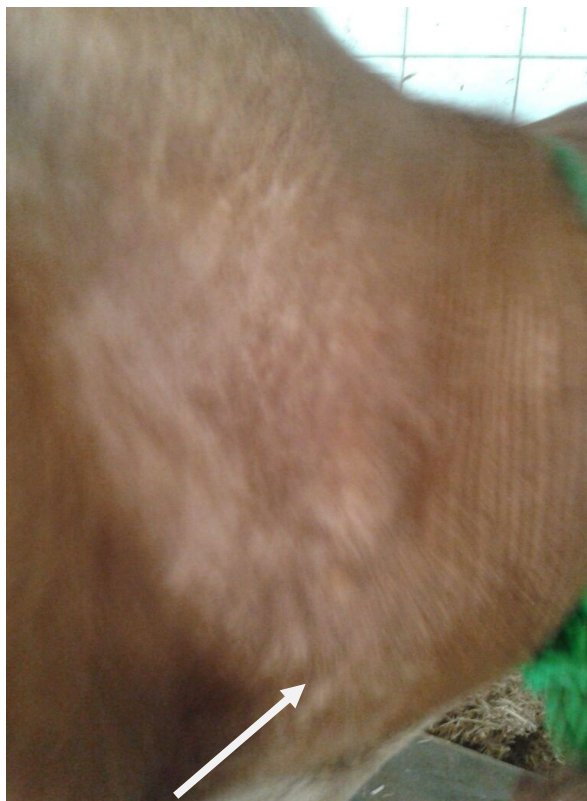


Photo (1): post vaccinal reaction in a vaccinated pregnant cow.

Serum neutralization test and Indirect ELISA of collected serum samples from pregnant cows vaccinated with the prepared LSD vaccine:

As shown in (Table 2) and Fig. (2 , 3) the results of LSD SNT and Indirect ELISA applied on collected serum samples from vaccinated pregnant cows showed protective antibody titer expressed as (1.5) NI and (1.32) S/P at 2 weeks post vaccination, the peak of antibody level was observed at 4th week post vaccination as (3.25) NI and (2.35) S/P. At 8th week post vaccination (at parturition time), there was decrease in the antibody level (2.25) NI and (1.96) S/P and the level of antibody continue to be protectable level till the end of the study (2.0) NI and (1.50) S/P.

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Table (2): Mean of NI and ELISA S/P ratio of collected sera samples from pregnant vaccinated cows.

Weeks post vaccination	mean NI ^{*(1)}		Mean S/P ^{*(2)}	
	Vaccinated pregnant cows	Control non-vaccinated pregnant cows	Vaccinated pregnant cows	Control non-vaccinated pregnant cows
0	0.50	0.25	0.08	0.04
1	0.50	0.50	0.64	0.06
2	1.50	0.50	1.32	0.05
3	2.50	0.25	1.76	0.08
^{(3)*} 4	3.25	0.50	2.35	0.09
5	3.25	0.25	2.33	0.06
6	3.00	0.50	2.30	0.04
7	3.00	0.50	2.34	0.05
^{(4)*} 8	2.25	0.25	1.96	0.06
9	2.25	0.50	1.84	0.06
10	2.25	0.50	1.79	0.07
11	2.25	0.25	1.72	0.09
12	2.00	0.25	1.68	0.08
14	2.00	0.50	1.61	0.05
16	2.00	0.50	1.57	0.06
18	2.00	0.50	1.52	0.07
^{(5)*} 20	2.00	0.25	1.53	0.05
24	2.00	0.50	1.50	0.09

NI = Neutralizing index.

S/P = Sample / positive ratio.

^{(1)*}NI ≥ 1.5 is considered protective (Cottral 1978).

^{(2)*}S/P >1.0 is considered protective (Babiuk et al. 2009).

^{(3)*} = Peak antibody levels.

^{(4)*}= parturition time.

^{(5)*}= weaning time

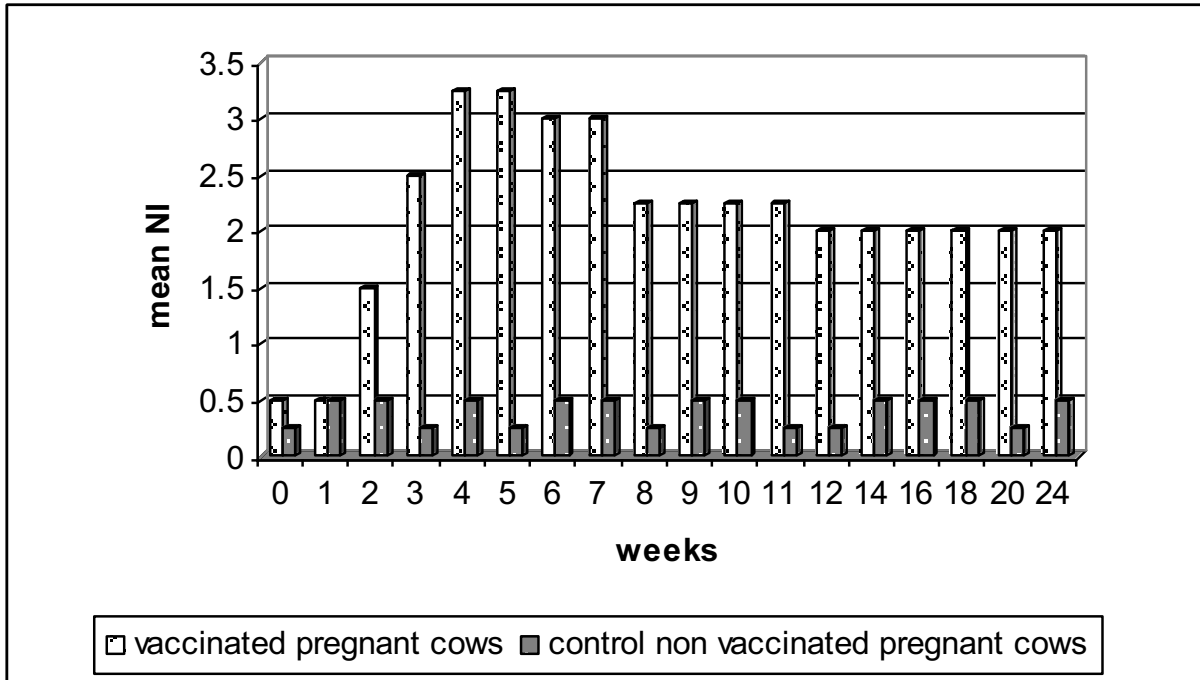


Fig. (2): Mean antibody neutralizing index of vaccinated pregnant cows compare to control non-vaccinated cows.

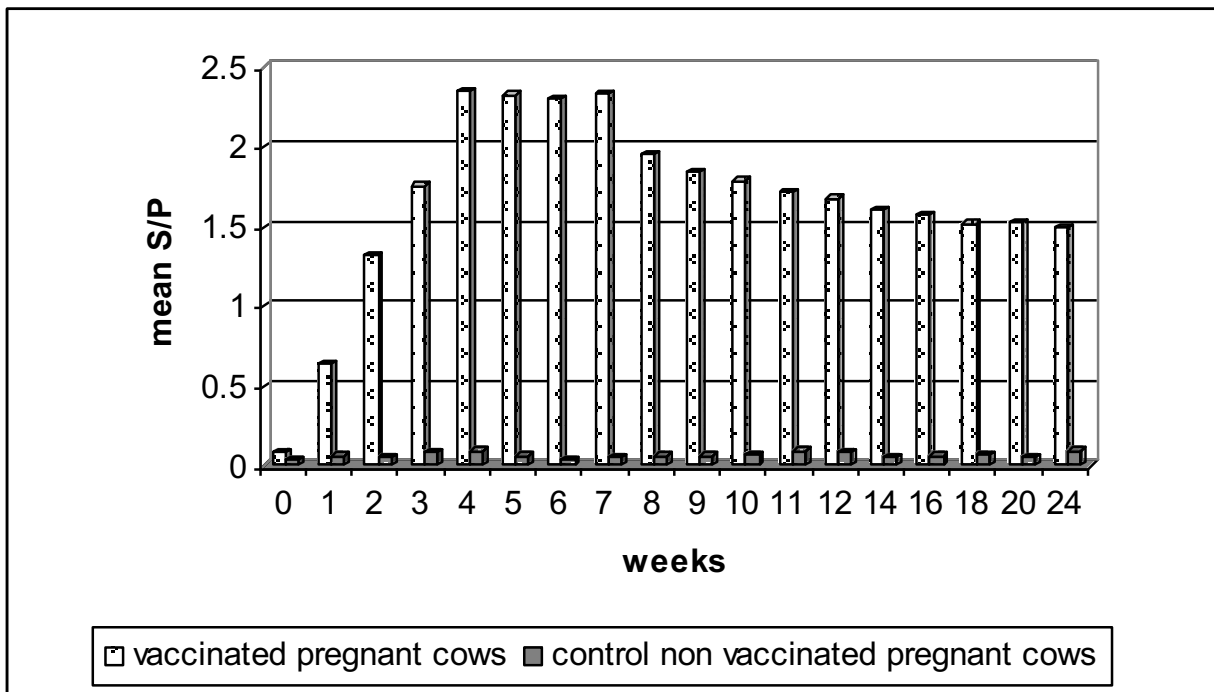


Fig. (3): Mean sample/positive ratio of vaccinated pregnant cows compare to control non-vaccinated cows.

Serum neutralization test and Indirect ELISA on serum samples collected from newly born calves from dams vaccinated with the prepared LSD vaccine:

As shown in (Table 3) and Fig. (4 and 5) the results of SNT and indirect ELISA of collected serum samples obtained from calves born from dams vaccinated with the experimentally prepared LSD vaccine showed protective antibody level from the parturition time (2.25) NI and (1.86) S/P, the level of antibody remain protective till 6 month post parturition as (1.5) NI and (1.11) S/P and become unprotective antibody level at the 7th month post parturition.

Table (3): Mean of NI and S/P ratio of serum samples collected from calves born from vaccinated dams

Age / month	Mean NI of		Mean S/P ratio	
	Calves born to vaccinated dams	Calves born to unvaccinated dams	Calves born to vaccinated dams	Calves born to unvaccinated dams
1 st week	2.50	0.25*	1.91	0.03*
1	2.25	0.25*	1.86	0.04*
2	2.00	0.50*	1.72	0.06*
3	2.00	0.50*	1.61	0.07*
4	1.75	0.25*	1.47	0.05*
5	1.75	0.50*	1.31	0.05*
6	1.50	0.50*	1.11	0.05*
7	1.25*	0.25*	0.93*	0.07*

NI= Neutralization index. S/P= sample to positive ratio.

NI ≥ 1.5 is considered protective (Cottral 1978).

S/P > 1.0 is considered protective (Babiuk et al. 2009).

* = susceptible animals.

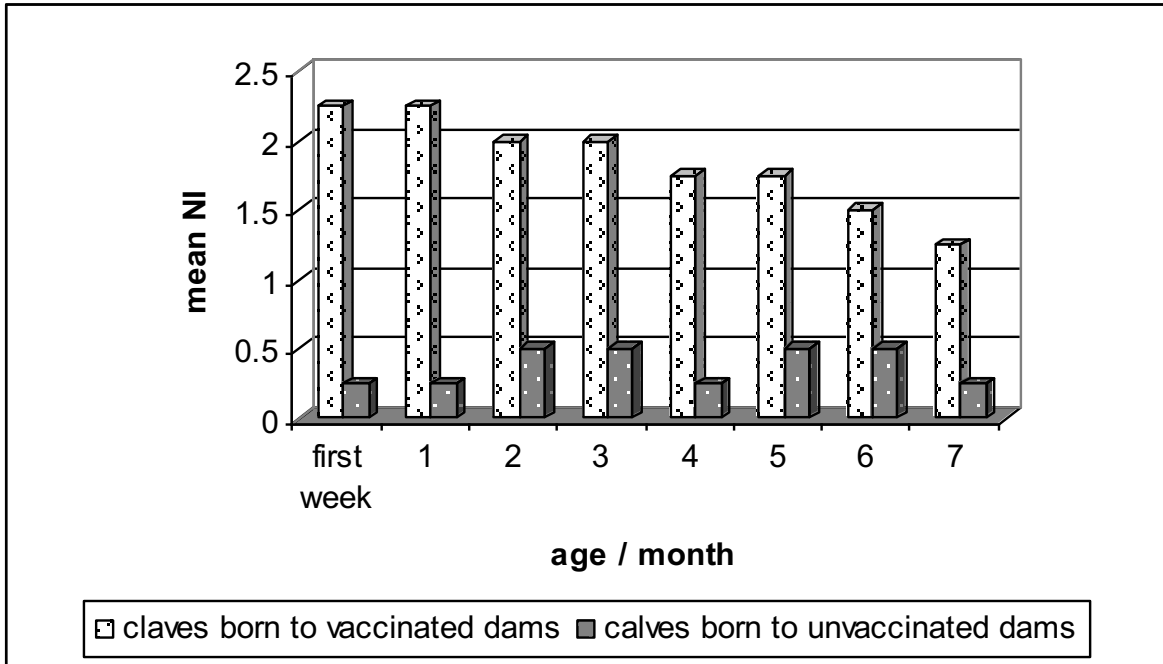


Fig. (4): Mean NI of serum samples collected from calves born from vaccinated dams compare to calves born from non-vaccinated dams.

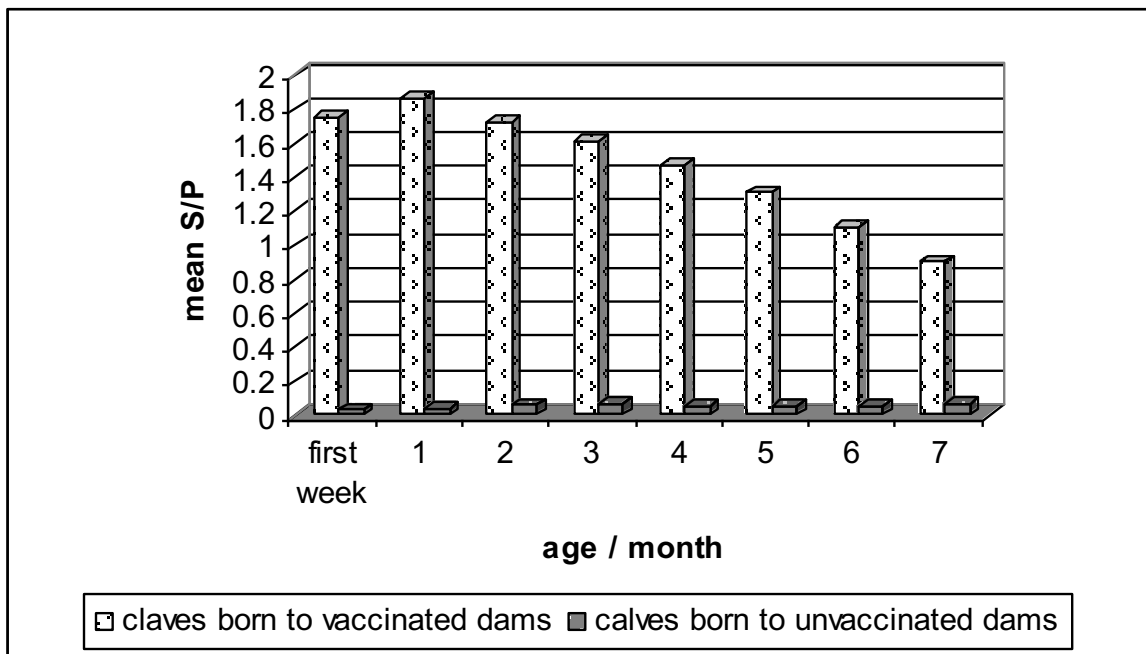


Fig. (5): Mean S/P ratio of serum samples collected from calves born from vaccinated dams compare to calves born from non-vaccinated dams.

DISCUSSION

Lumpy skin disease (LSD) is an important infectious viral disease of cattle causing high economic losses. In Egypt, vaccination with sheep pox vaccine (SPV) is the only method to control the disease (**Agag et al., 1994**). In this study the immune response of experimentally prepared live attenuated LSD vaccine was studied in pregnant cows and their offspring calves. Clinical examination of pregnant cows vaccinated with prepared LSD vaccine showed a local reaction at the point of the inoculation which disappeared within 12-15 days, in addition to slight increase in body temperature of vaccinated cows beginning at the 4th day post vaccination, it remained within the temperature range of vaccinated cows (38.3 -39.3) for 8 days post vaccination as **Woods, (1988)** **Carn, (1993)** and **Aboul- Soud, (1996)**, stated that the skin reaction and slight rise in temperature indicated that the vaccine stimulated the immune system in the susceptible animal. There was no abortion recorded, no congenital abnormalities in new born calves, and no symptoms for LSD appeared even in 10x field dose inoculated cows which indicated that the vaccine was safe. These results are in accordance with **Code of federal Regulation (2005)** and **OIE (2012)** which reported the parameters of the safe produced vaccine as should gave good satisfactory result of safety. The results were in accordance with **Heba, (2016)** who recorded no abortion in the pregnant cows vaccinated with LSD vaccine. Evaluation of humoral immune response depending mainly on the antibody titers in sera of vaccinated dams by using SNT and ELISA as **Davies and Otema (1981)** who employed SNT to assess the post vaccinal immune status. The result of SNT and ELISA in pregnant cows vaccinated with prepared LSD vaccine reached the protective level at the second week post vaccination and increased gradually till reached its highest level at 4th week post vaccination. The SNT and ELISA results indicated that the prepared LSD vaccine is immunogenic and protective till the end of the experiment as agreed with **OIE, (2010)** that reported neutralizing index (NI) ≥ 1.5 is considered protective against capripox viruses. Also, **Agag et al., (1992)** mentioned that serum neutralizing antibodies develop on the 2nd week and a significant rise of antibody titer was recorded from the 21th to 42th days post inoculation. The results of SNT and ELISA also were in accordance with **Amal et al., (2007)** who mentioned that calves vaccinated with prepared LSD vaccine reached protective level of **NI (1.5)** at the second week post vaccination and increased gradually till reach (3.1) at 4weeks post vaccination then reached to (1.5) at 40 weeks from vaccination.

Investigation of serum antibody titers in newly born calves from vaccinated dams by using SNT and ELISA showed that, the NI and S/P of newly born calves were protected from the first week after parturition (2.25 NI and 1.86 S/P) and persisted in protective level until 6 months age as (1.5) NI and (1.11) S/P respectively then decrease than the protective level. These results is supported by **Kitching, (2003)** who confirmed transfer of maternal antibodies from dams vaccinated with LSDV vaccine to newly born calves via colostrum, and **Woods, (1988)** who mentioned that passive immunity from vaccinated dams might interfere with the efficient vaccination of calves less than 6 months old. In conclusion, this study clearly showed that Lumpy Skin Disease vaccine was highly immunogenic and safe in pregnant cows and gave protective maternal immunity for calves born from vaccinated cows persist up to 6 months which considered it a good choice vaccine to control LSD in the field.

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متابعه تاثير لقاح تجريبي لفيروس الجلد العقدي على الابقار العشار

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المعمل المركزى للرقابه على المستحضرات الحيويه البيطريه**

المستشفى البيطرى العسكرى بالقاهره***

الملخص العربى

تم فى هذه الدراسه تتبع رده الفعل والمناعه المكتسبه فى الابقار العشار المحصنه بلقاح الجلد العقدي الحى المستضعف حيث تم تحصين ثمانى ابقار عشار بالجرعة الحقلية من اللقاح فى ادمه الجلد وبقرتين عشار بعشرة اضعاف الجرعة الحقلية مع متابعه رده الفعل ودرجات الحراره طوال 14 يوم بعد التحصين. وقد اظهرت الابقار المحصنه ارتفاع فى درجه الحراره بدأ من اليوم الرابع (38.8o) ثم عاودت الهبوط الى المستويات الطبيعيه فى اليوم التاسع (38.1o م). كما لوحظ تفاعل موضعى فى مكان الحقن عند اليوم الرابع اختلفى تدرجيا بعد خمسه عشار يوما. هذا ولم تسجل اى حالات اجهاض او ظهور لإعراض مرض الجلد العقدي فى الابقار العشار المحصنه بالجرعة الحقلية او عشر اضعاف الجرعة الحقلية.

تم عمل التتبع المناعى لعينات المصل المأخوذه من الابقار المحصنه عن طريق اختبارى التعادل المصلى والاليزا حيث بلغت المستويات المناعيه الوقائيه للاختبارين (1.5) و (1.32) لمؤشر التعادل ونسبه S/P على الترتيب عند الاسبوع الثانى وارتفعت المستويات تدرجيا الى ان بلغت اعلى مستوى لها (3.25) و (2.35) على الترتيب عند الاسبوع الرابع ثم انخفضت تدرجيا الى (2.00) و (1.50) على الترتيب حتى الاسبوع الرابع والعشرون من التحصين.

كما تم فى هذه الدراسه دراسه المستوى المناعى لعينات السيرم المأخوذه من العجول المولوده من الابقار المحصنه حيث بلغت قيم التعادل المصلى والاليزا (2.25) و (1.86) للاختبارين على الترتيب من الاسبوع الاول من الولاده. وظل المستوى المناعى وقائى حتى الشهر السادس من الولاده (1.5) و (1.11) على الترتيب ثم انخفضت الى مستويات غير وقائيه بعد الشهر السادس.

مما سبق يمكن القول ان لقاح الجلد العقدي المستضعف امن وينتج عنه مستوى مناعه عالي لفته طويله فى الابقار العشار كما ان المناعه الاميه المكتسبه والمنقوله الى العجول المولوده من الامهات المحصنه كانت كافيه للوقايه من المرض لمدته سته اشهر.